Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis

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Caspase-1 is activated by a variety of stimuli after the assembly of the "inflammasome," an activating platform made up of a complex of the NOD-LRR family of proteins. Caspase-1 is required for the secretion of proinflammatory cytokines, such as interleukin (IL)-1β and IL-18, and is involved in the control of many bacterial infections. Paradoxically, however, its absence has been reported to confer resistance to oral infection by *Salmonella typhimurium*. We show here that absence of caspase-1 or components of the inflammasome does not result in resistance to oral infection by *S. typhimurium*, but rather, leads to increased susceptibility to infection.

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Caspases are evolutionary conserved cysteine proteases with a very important role in apoptosis (1). Caspase-1, however, is best known for its role in inflammation and innate immune responses to microbial pathogens (2). Caspase-1 activation takes place upon assembly of the "inflammasome," a signaling platform scaffolded by proteins belonging to the NOD-LRR family (2). Activated caspase-1 is responsible for the processing and maturation of the proinflammatory cytokines IL-1β and IL-18 (3), which are important in host defense against a variety of pathogens. Consequently, caspase-1–deficient mice are more susceptible to several pathogens (4–6) but more resistant to the toxic effect of lipopolysaccharide (7).

Surprisingly, caspase-1 deficiency was shown to confer resistance to oral infection with the enteropathogen *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) (8). This bacterium induces caspase-1–dependent death of macrophages upon infection (9–11). This type of death differs from conventional apoptosis in that it occurs very rapidly and does not exhibit some of its canonical features. Because in activated macrophages this rapid death apparently occurs with release of proinflammatory cytokines, it was proposed that caspase-1 was required for *S. typhimurium* to colonize the Peyer's patches (PPs) and cross the intestinal barrier (8).

The caspase-1–dependent macrophage death induced by *Salmonella* is dependent on the function of a type III secretion system (TTSS) encoded within its pathogenicity island-1 (SPI-1) (9, 10). It has been proposed that SipB, one of the proteins secreted and translocated into host cells by this TTSS, is responsible for this death by directly binding and activating caspase-1 (12). However, because *ipaf*$^{-/-}$ macrophages are also resistant to this death (13), caspase-1 activation by *S. typhimurium* is most likely not mediated by direct binding of SipB to caspase-1. *Shigella flexneri*, another enteric pathogen, causes a virtually identical caspase-1–dependent macrophage death (14). Paradoxically, caspase-1 deficiency renders mice significantly more susceptible to infection with this pathogen (4). Furthermore, administration of IL-18 or IL-1β or neutralizing antibodies to these cytokines increases the resistance or susceptibility of mice to *S. typhimurium* infection, respectively (15–18). These apparent paradoxes prompted us to reexamine the role of caspase-1 in *S. typhimurium* pathogenesis.

RESULTS AND DISCUSSION

We first reexamined the role of caspase-1 in controlling *S. typhimurium* infection. We first

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confirmed the genotype of *caspase-1*$^{-/-}$ mice by PCR genotyping (not depicted) and, functionally, by the resistance of their bone marrow–derived macrophages (BMDMs) to the rapid death induced by *S. typhimurium* (Fig. 1 A). The mouse *nramp1* (Slc11a1) gene controls the resistance or susceptibility of mice to several unrelated pathogens, among them *S. typhimurium* (19). Common laboratory mouse strains such as C57BL/6 and BALB/c carry a mutant *nramp1*, which renders them up to 1,000-fold more susceptible to *S. typhimurium* than strains harboring the wild-type allele (19). Knockout mouse lines are most frequently constructed using 129-derived embryonic stem (ES) cells, which have a wild-type *nramp1* allele. However, deficient animals are also frequently backcrossed into another genetic background, such as that of C57BL/6, with a mutant copy of *nramp1*. Given these considerations and because *nramp1* is the main genetic determinant of *S. typhimurium* susceptibility/resistance in mice, we determined the *nramp1* status of the control and knockout mice used in these studies. As seen in Fig. 1 B, mice carried a mutant copy of the *nramp1* gene and are therefore "*Salmonella* susceptible." We used these mice to reevaluate the influence of caspase-1 in susceptibility to *S. typhimurium* oral infection. *Caspase-1*$^{-/-}$ and control mice were infected orally with 10^5 *S. typhimurium* (~10 LD50 for an *nramp1*$^{-/-}$ mouse). As expected for this dose, all control mice died during the experiment. However, unlike previously reported, *caspase-1*$^{-/-}$ mice did not show increased resistance to bacterial infection and succumbed to infection more rapidly than the *caspase-1*$^{+/+}$ control animals (Fig. 1 C). These results indicate that in the context of Nramp-1 deficiency, the absence of caspase-1 does not confer resistance to *S. typhimurium* oral infection, but rather, increases the susceptibility to this pathogen. We also quantified the bacterial loads in the spleens and mesenteric LNs (MLNs) of mice 5 d after oral administration of 10^6 *S. typhimurium*. Consistent with the previous result, significantly

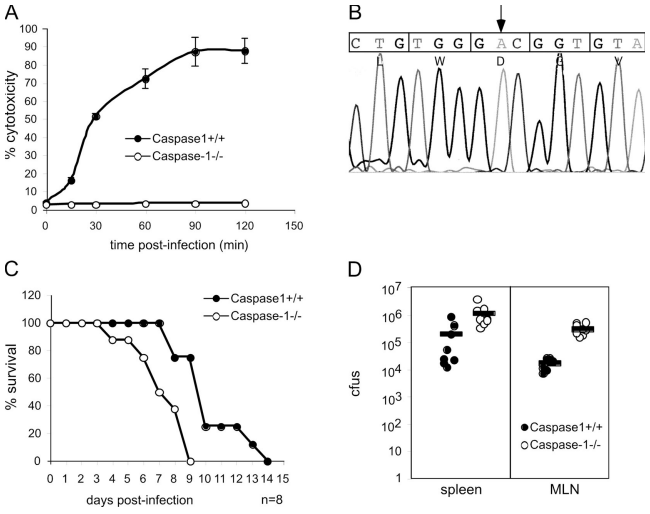

Figure 1. Susceptibility of *caspase-1*$^{-/-}$*nramp-1*$^{-/-}$ mice to *S. typhimurium* infection. (A) To confirm the *caspase-1*$^{-/-}$ status, which confers resistance to *Salmonella*-induced macrophage death, BMDMs obtained from *caspase-1*$^{+/+}$*nramp-1*$^{-/-}$ and *caspase-1*$^{-/-}$*nramp-1*$^{-/-}$ mice were infected with *S. typhimurium*, and macrophage death was evaluated by the release of LDH. (B) Nucleotide sequence profile of the *nramp-1* allele. The arrow indicates the presence of the mutation G to A leading to the change of a glycine for aspartic acid at position 169, which results in a loss of function. (C) Mice were infected orally with 10^5 *S. typhimurium*, and survival was monitored over time. (D) Mice were infected orally with 10^6 *S. typhimurium*, and bacterial loads in the spleens and MLNs were determined 5 d after infection (p-values for the difference in bacterial loads in *caspase-1*$^{-/-}$*nramp-1*$^{-/-}$ and *caspase-1*$^{+/+}$*nramp-1*$^{-/-}$ mice were $P = 0.02$ and $P < 0.0001$ for the spleens and MLNs, respectively).

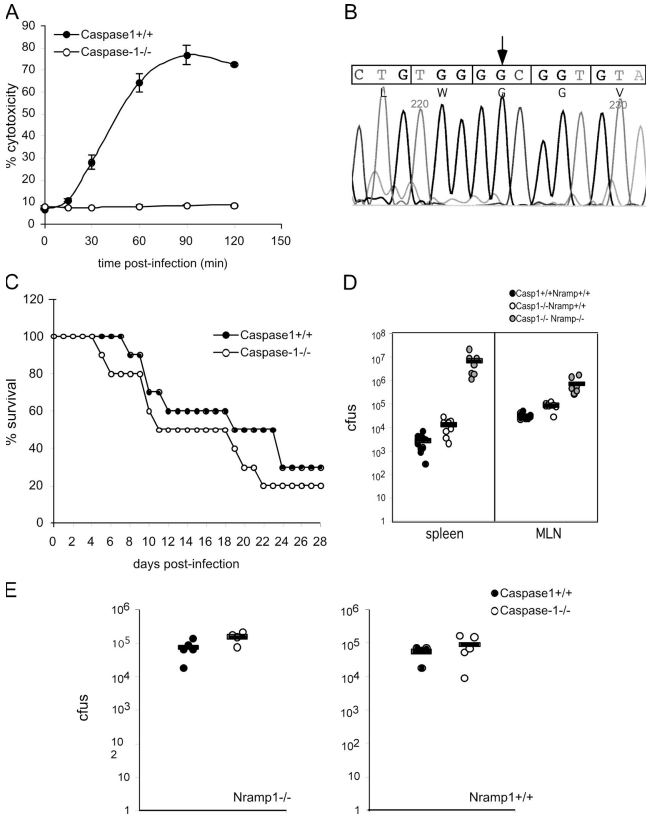

Figure 2. Susceptibility of *caspase-1*$^{-/-}$*nramp-1*$^{+/+}$ mice to *S. typhimurium* infection. (A) To confirm the *caspase-1*$^{-/-}$ status, BMDMs obtained from *caspase-1*$^{+/+}$*nramp-1*$^{+/+}$ and *caspase-1*$^{-/-}$*nramp-1*$^{+/+}$ mice were infected with *S. typhimurium*, and macrophage death was evaluated by the release of LDH. (B) Nucleotide sequence confirmed the presence of the wild-type *nramp1* allele. The arrow indicates a wild-type codon at position 169 (see Fig.1). (C) Mice were infected orally with 10^6 *S. typhimurium*, and survival was monitored over time. (D) Mice were infected orally with 10^7 *S. typhimurium*, and bacterial loads in the spleens and MLNs were determined 5 d after infection (p-values for the difference in bacterial loads in *caspase-1*$^{-/-}$*nramp-1*$^{+/+}$ and *caspase-1*$^{+/+}$*nramp-1*$^{+/+}$ mice were $P = 0.0016$ and $P < 0.0001$ for the spleens and MLNs, respectively). (E) Mice were infected orally with 10^6 (*nramp-1*$^{-/-}$ background) or 10^7 (*nramp-1*$^{+/+}$ background) *S. typhimurium*, and bacterial loads in PPs were determined 5 d after infection (p-values for the difference in bacterial loads between *caspase-1*$^{+/+}$*nramp-1*$^{-/-}$ vs. *caspase-1*$^{-/-}$*nramp-1*$^{-/-}$ and between *caspase-1*$^{+/+}$*nramp-1*$^{+/+}$ vs. *caspase-1*$^{-/-}$*nramp-1*$^{+/+}$ mice were $P = 0.114$ and $P = 0.222$, respectively).

higher numbers of bacteria were present in the organs of *caspase-1*$^{-/-}$ mice ($P = 0.002$ and $P < 0.0001$ for spleens and MLNs, respectively; Fig. 1 D). Collectively, these results indicate that the absence of caspase-1 in the context of a nonfunctional *nramp1* allele does not result in increased resistance to orally administered *S. typhimurium*. The observation of higher bacterial loads in systemic tissues of the *caspase-1*$^{-/-}$–deficient animals indicates that caspase-1 is most likely not required for these bacteria to cross the intestinal barrier as reported previously (8). It is not clear why these results differ from the previous report. It is possible that the different results may be due to the use of a different caspase-1–deficient line because the previous studies were conducted with an independently generated *caspase-1*$^{-/-}$ mouse in a B10.RIII background. Previous studies have reported that this background exhibits a more pronounced Th1 polarization of the immune response (20). However, more studies will be necessary to determine the bases for the differences in the findings.

To investigate whether the phenotype associated with the absence of caspase-1 could be masked by the absence of a functional *nramp1* allele, we crossed the wild-type *nramp1* allele into the *caspase-1*$^{-/-}$ background. The *caspase-1*$^{-/-}$ genotype of the resulting mice was confirmed by PCR genotyping (not depicted) and by the inability of wild-type *S. typhimurium* to kill *caspase-1*$^{-/-}$ BMDMs (Fig. 2 A). The *nramp1*$^{+/+}$ status of the mice was confirmed by sequencing (Fig. 2 B). *Caspase-1*$^{-/-}$*nramp1*$^{+/+}$ mice were then infected orally with 10^6 wild-type *S. typhimurium* ($\sim$10 LD50 for an *nramp1*$^{+/+}$ mouse). As in the case of the *nramp1*$^{-/-}$ background, the absence of caspase-1 in the context of a wild-type allele of *nramp1* also resulted in increased susceptibility to *S. typhimurium* infection as shown by the shorter time to death (Fig. 2 C). Consistent with these results, bacterial loads recovered from the spleens and MLNs 5 d after infection were significantly higher in *caspase-1*$^{-/-}$*nramp1*$^{+/+}$ when compared with the controls ($P = 0.0016$ and $P < 0.0001$ for spleens and MLNs, respectively; Fig. 2 D). Bacterial loads in mice carrying both mutations (i.e., *caspase1*$^{-/-}$*nramp1*$^{-/-}$) were much higher than in mice carrying a mutation in either allele (Fig. 2 D), indicating that Nramp1 and caspase-1 operate in independent defense pathways against *S. typhimurium*. These experiments demonstrate that the ability of *S. typhimurium* to kill macrophages in a caspase-1–dependent manner is not required for bacteria to cross the intestinal barrier and become systemic, and that caspase-1 deficiency leads to an increased susceptibility to infection.

Previous studies have shown that the SPI-1 TTSS plays a very important role in the colonization of PPs before its translocation to systemic sites (21, 22). To investigate whether the SPI-1 TTSS– and caspase-1–dependent macrophage death was important for *S. typhimurium* colonization of PPs, we compared the bacterial loads at this site after infection of wild-type and *caspase1*$^{-/-}$ mice. We found that absence of caspase-1 did not decrease but slightly increased ($P = 0.114$ and $P = 0.259$ in *nramp1*$^{-/-}$ and *nramp1*$^{+/+}$ backgrounds, respectively) the bacterial loads in PPs in both *nramp1*$^{-/-}$ and *nramp1*$^{+/+}$ backgrounds (Fig. 2 E), further supporting the conclusion that translocation of *S. typhimurium* to this site does not require the SPI-1 TTSS–mediated caspase-1–dependent macrophage death.

In the mouse, *S. typhimurium* causes a systemic disease that more closely resembles typhoid fever than gastroenteritis, the syndrome that this bacterium most often causes in humans. However, in mice pretreated with antibiotics, *S. typhimurium* causes acute colitis, which has been used as a model for the study of *S. typhimurium*–induced intestinal inflammation (23). Previous studies have demonstrated that the SPI-1–encoded TTSS plays an important role in the ability of *S. typhimurium* to induce colitis in the streptomycin-treated mouse model (24). However, it is unknown whether the ability of *S. typhimurium* to rapidly kill macrophages through the activity of its SPI-1–encoded TTSS plays a role in the induction of inflammation in this model system. We therefore compared the ability of *S. typhimurium* to induce colitis in wild-type and caspase-1–deficient mice because the SPI-1–dependent *S. typhimurium*–induced macrophage death is dependent on caspase-1. Streptomycin-pretreated *caspase-1*$^{-/-}$ and control mice were orally infected with *S. typhimurium*, and 48 h after infection, ceca were removed for histopathological analysis. Streptomycin-treated uninfected mice exhibited normal histology of the intestinal epithelium (Fig. 3 A). In contrast, streptomycin-treated *S. typhimurium*–infected *caspase-1*$^{+/+}$ mice showed marked inflammation of the intestinal epithelium (Fig. 3 A). The cecum histopathology of the infected animals was characterized by profuse mucosal edema, marked infiltration of neutrophils, and substantial disruption of the epithelium. *caspase-1*$^{-/-}$ mice showed even more pronounced inflammation than control mice (Fig. 3 A). The lesions in the *caspase-1*$^{-/-}$ mice showed more edema, more neutrophil infiltration, and epithelial disruption. The more pronounced histopathological changes could not be ascribed to higher bacterial burden because there was not a significant difference in the bacterial loads recovered from this tissue in both types of animals (Fig. 3 B). Rather, the more pronounced histopathology may be the result of *S. typhimurium* reaching deeper tissues in the absence of caspase-1. These results indicate that the ability of *S. typhimurium* to cause inflammation in the cecum is largely independent of its ability to activate caspase-1. Furthermore, contrary to what has been reported previously (8), the ability of *S. typhimurium* to rapidly kill macrophages in a SPI-1 TTSS– and caspase-1–dependent manner does not seem to contribute significantly to its ability to cause colitis. Because the SPI-1 TTSS has been shown to be essential for *S. typhimurium* to cause colitis (24), activities mediated by this TTSS other than macrophage killing must be more important for the stimulation of the inflammatory response.

Caspase-1 is activated upon assembly of an intracellular complex known as the inflammasome. IPAF and NALP3 are key components of this multi-protein complex (2, 25). IPAF is a CARD-containing protein and has been shown to bind the CARD domain of caspase-1 resulting in caspase-1 activation

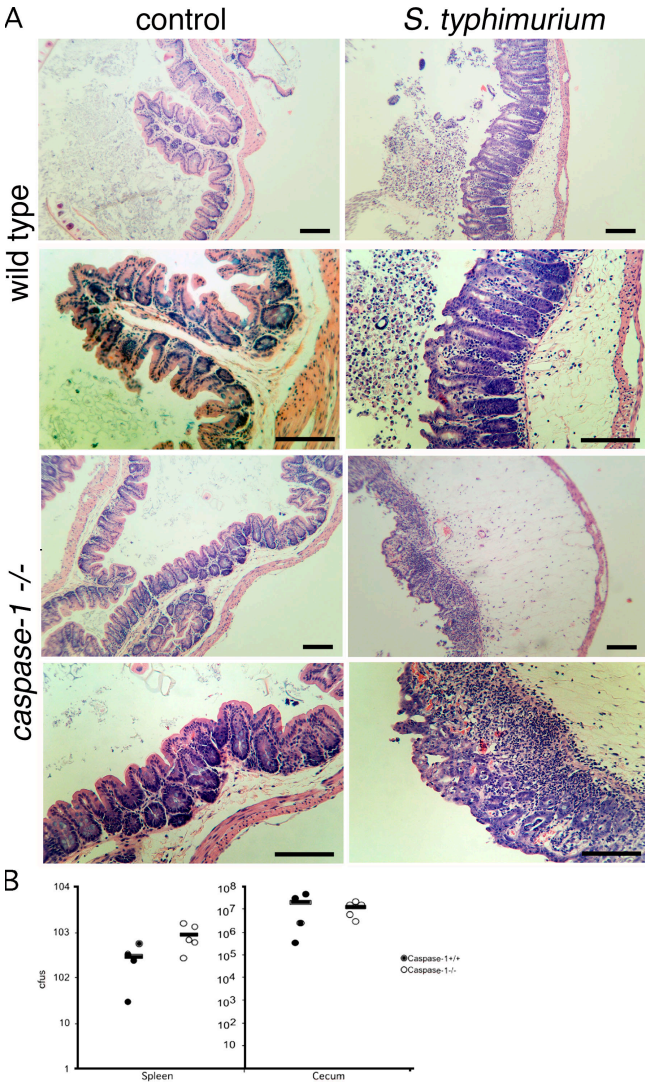

Figure 3. Histopathology of ceca from streptomycin-treated wild-type and *caspase-1*$^{-/-}$ mice orally infected with *S. typhimurium*. (A) Wild-type and *caspase-1*$^{-/-}$ mice (*nramp-1*$^{+/+}$ background) were treated with 20 mg streptomycin, and 24 h after antibiotic treatment, mice were either mock infected (control) or infected orally with 10^8 *S. typhimurium*. 48 h after infection, ceca were removed, fixed, and embedded in paraffin, and tissue sections were stained with hematoxylin and eosin. Similar results were obtained in three independent animals for each group. (B) Mice were treated and infected with *S. typhimurium* as indicated above, and bacterial loads in the spleens and ceca were determined 48 h after infection (p-values for the difference between the bacterial loads in the spleens and ceca of *caspase-1*$^{-/-}$ vs. *caspase-1*$^{+/+}$ mice were P = 0.138 and P = 0.483, respectively).

(26). NALP3 activates caspase-1 through the adaptor molecule ASC (apoptosis-associated speck-like protein containing a CARD) (27). ASC is essential for the caspase-1 activation mediated by several pathogen-associated molecular patterns (13, 28). Recent studies have also shown that *S. typhimurium* can kill *asc*$^{-/-}$ BMDMs, although it does so with delayed kinetics, indicating that ASC is not essential for *S. typhimurium* TTSS-dependent caspase-1 activation (13). In contrast, *ipaf*$^{-/-}$ BMDMs failed to show activation of caspase-1 upon bacterial infection and were as resistant to *S. typhimurium*–induced death as *caspase-1*$^{-/-}$ BMDMs (13). These results suggest that IPAF and caspase-1 are in the same pathway of bacterial-induced death. However, *ipaf*$^{-/-}$ BMDMs did not show any defect in caspase-1 activation when stimulated with pathogen-associated molecular patterns and ATP (13), indicating the existence of alternative pathways of caspase-1 activation. NALP3, another component of the inflammasome, has been implicated in the ASC-dependent activation of caspase-1 (29). However, *nalp3*$^{-/-}$ BMDMs were fully susceptible to the *Salmonella*-induced death (30, 31). Collectively, these results suggest the existence of at least two different pathways leading to caspase-1 activation, one mediated by NALP3 and the adaptor molecule ASC (resulting in proinflammatory cytokine production) and the other mediated by IPAF (resulting in cell death). Whether the different outputs are the result of different levels of caspase-1 activation or the result of the recruitment of different caspase-1 effectors is unclear.

Given the observation that *S. typhimurium* is capable of activating the inflammasome and caspase-1 by different pathways, we examined the role of different components of the inflammasome in *S. typhimurium* pathogenesis. *nalp3*$^{+/+}$ and *nalp3*$^{-/-}$ mice (carrying the *nramp1* mutant allele) were infected orally with 10^5 wild-type *S. typhimurium*, and survival was monitored daily. Both *nalp3*$^{+/+}$ and *nalp3*$^{-/-}$ succumbed to bacterial infection within the same time frame (Fig. 4 A) and exhibited similar bacterial loads in the spleens and MLNs 5 d after infection (Fig. 4 B). We also examined the susceptibility of *asc*$^{-/-}$ mice to *S. typhimurium* infection. Similar to *nalp3*$^{-/-}$ mice, ASC deficiency did not change the susceptibility to *S. typhimurium* infection as measured by time to death and bacterial loads in different tissues (Fig. 4, C and D). Because the absence of caspase-1 resulted in increased susceptibility to *S. typhimurium* infection (Figs. 1 and 2), these results suggest that *S. typhimurium* must be able to activate caspase-1 independently of NALP3 and ASC. Previous studies have also shown that IPAF-deficient macrophages are resistant to *S. typhimurium*–induced death, suggesting an important role for IPAF in *S. typhimurium*–induced caspase-1 activation (13). Therefore, we examined the susceptibility of *ipaf*$^{-/-}$ mice to oral *S. typhimurium*. *ipaf*$^{+/+}$ and *ipaf*$^{-/-}$ mice (carrying the *nramp1* mutant allele) were infected orally with 10^6 *S. typhimurium*, and the bacterial loads in the spleens and MLNs 5 d after infection were determined. IPAF deficiency did not increase resistance to *S. typhimurium* infection (Fig. 4 E). In contrast, the bacterial loads were slightly higher in the *ipaf*$^{-/-}$ mice, although the difference was not statistically significant. The observation that the bacterial loads in systemic tissues were not reduced in the *ipaf*$^{-/-}$ mice further supports the notion that the IPAF- and caspase-1–dependent *Salmonella*-induced macrophage death is not required for these bacteria to cross the intestinal barrier.

Collectively, these results, combined with previously published reports (13, 30, 31), indicate that *S. typhimurium*

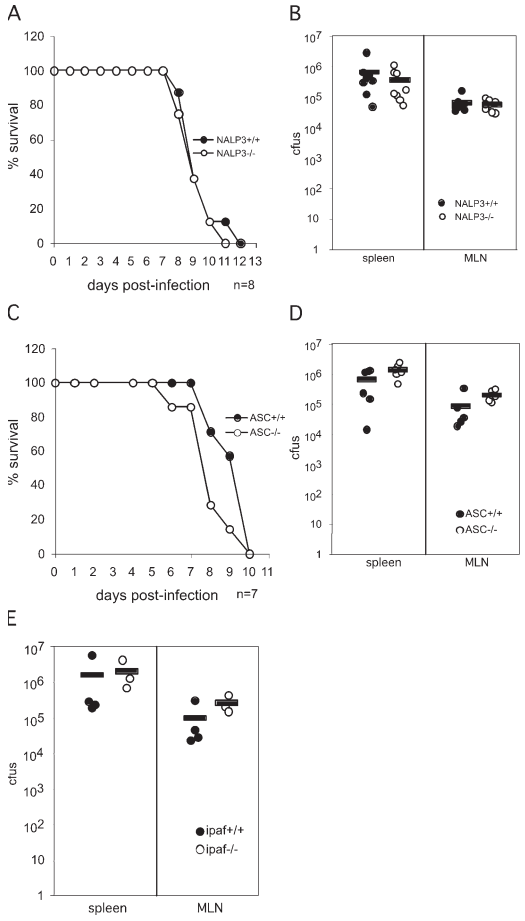

Figure 4. Susceptibility to *S. typhimurium* infection of mice deficient in essential components of the inflammasome. (A) *nalp3*$^{+/+}$ and *nalp3*$^{-/-}$ mice were infected orally with 10^5 wild-type *S. typhimurium*, and survival was monitored over time. (B) *nalp3*$^{+/+}$ and *nalp3*$^{-/-}$ mice were infected orally with 10^6 *S. typhimurium*, and the bacterial loads in the spleens and MLNs were determined 5 d after infection. (C) *asc*$^{+/+}$ and *asc*$^{-/-}$ mice were infected orally with 10^5 wild-type *S. typhimurium*, and survival was monitored over time. (D) *asc*$^{+/+}$ and *asc*$^{-/-}$ mice were infected orally with 10^6 *S. typhimurium*, and the bacterial loads in the spleens and MLNs were determined 5 d after infection. (E) *ipaf*$^{+/+}$ and *ipaf*$^{-/-}$ mice were infected orally with 10^6 *S. typhimurium*, and the bacterial loads in the spleens and MLNs were determined 5 d after infection.

possesses different redundant mechanisms to activate caspase-1 through different components of the inflammasome. Furthermore, these results indicate that contrary to what has been proposed previously, the absence of caspase-1 does not result in resistance to *S. typhimurium* infection and that this pathogen does not require the activity of this enzyme to cross the intestinal barrier.

MATERIALS AND METHODS

Mouse strains. The *caspase-1*$^{-/-}$, *nalp3*$^{-/-}$, and *asc*$^{-/-}$ mouse lines have been described previously (7, 30) and were backcrossed to the C57BL/6 background for five (*caspase-1*$^{-/-}$ and *nalp3*$^{-/-}$) and six (*asc*$^{-/-}$) generations. To generate *ipaf*$^{-/-}$ mice, an IPAF targeting vector (Fig. S1 A, available at http://www.jem.org/cgi/content/full/jem.20060206/DC1) was electroporated into 129SvEvBrd Lex-1 ES cells. Homologous recombinant ES cells were identified by Southern blot analysis and microinjected into C57BL/6 blastocysts. Chimeric offspring were backcrossed to C57BL/6 mice, and germline transmission was confirmed by PCR of tail genomic DNA (Fig. S1 B). Screening of IPAF mice using the primers (no. 28) 5′-GCAGGAATCAATCCAGAGTCTGAG and (no. 37) 5′-GAAGCCTCAACGGCAACGAGCACTC amplify a 744-bp product from the wild-type allele. Primers (no. 37) 5′-GAAGCCTCAACGGCAACGAGCACTC and (Neo3A) 5′-GCAGCGCATCGCCTTCTATC amplify a 222-bp product from the targeted allele. RT-PCR analysis of cDNA isolated from *ipaf*$^{+/+}$, *ipaf*$^{+/-}$, and *ipaf*$^{-/-}$ splenocytes (Fig. S1 C) confirmed the absence of IPAF mRNA in *ipaf*$^{-/-}$ mice. Primers used for RT-PCR analysis were as follows: GAPDH, TCACCACCATGGAGAAGGC and GCTAAGCAGTTGGTGGTGCA; IPAF, ATGTCATTTACTGTGAGCCCTTGG and TTGCCAGACTCGCCTTCAATC. IPAF-deficient mice were backcrossed onto a C57BL/6 background for two generations. All mice were used at 8–12 wk of age. Wild-type littermates were used as controls.

Nramp1 genotyping. A 514-bp fragment of the *nramp1* gene was amplified by PCR using primers 5′-AAGTGACATCTCGCCATAGGTGCC-3′ and 5′-TTCTCTCACCATAGTTATCCAAG AAG-3′ (forward and reverse, respectively). The purified PCR product was then sequenced using the primer 5′-CCCCCATCTATGTTATCACCC-3′.

***S. typhimurium* strains.** *S. typhimurium* SL1344 was the wild-type strain used in all the experiments. SB161 and SL1344 derivative carrying a nonpolar mutation in the *invG* gene, which encodes an essential component of the SPI-1 TTSS, has been described previously (9). To prepare cultures for cytotoxicity assays or infection of mice, overnight cultures were subcultured for 4 h in LB medium containing 0.3 M NaCl to an OD_{600} of 0.9 to induce expression of the SPI-1–encoded TTSS.

Cytotoxicity assays. BMDMs were seeded on 24-well tissue culture dishes at 2×10^5 cells/well density. Before infection, the complete medium was replaced by serum-free HBSS. Bacteria grown as described above were added to wells at a multiplicity of infection of 20 bacteria/cell, and supernatant samples were collected at the indicated time points to be assayed for lactate dehydrogenase (LDH) activity as a marker for cell death. LDH assays were performed using the CytoTox 96 nonradioactive cytotoxicity assay (Promega).

Animal infection experiments. All animal experiments were conducted according to protocols approved by Yale University's Institutional Animal Care and Use Committee. Groups of age- and sex-matched mice were infected at 8–12 wk of age. After 8 h of fasting, mice were administered by intragastric gavage 100 μl of 10% bicarbonate solution followed by the indicated bacterial dose in 100 μl PBS. To determine bacterial loads in the spleens and MLNs, mice were killed 5 d after infection, organs were homogenized in 3 ml PBS containing 0.05% sodium deoxycholate, and dilutions were plated on LB plates containing streptomycin to determine colony-forming units.

***S. typhimurium*–induced colitis model in streptomycin-treated mice.** Bacterial infections in streptomycin-treated animals were performed essentially as described previously (23). In brief, mice (three per group) were treated orally with 20 mg streptomycin, and 24 h after antibiotic treatment, mice were infected orally with wild-type *S. typhimurium* as described above. Mice were killed 48 h after infection, and ceca were fixed in formalin for histopathology processing. Fixed tissues were embedded in paraffin, and tissue sections were stained with hematoxylin and eosin. In a parallel identical experiment, ceca were homogenized in 3 ml PBS containing 0.05% sodium deoxycholate and dilutions were plated on LB plates containing streptomycin to determine colony-forming units.

Online supplemental material. Fig. S1 shows the targeted disruption of the murine *IPAF* gene and is available at http://www.jem.org/cgi/content/full/jem.20060206/DC1.

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